CLAIMS

1. A method for classifying and counting leukocytes, which comprises:

(1) a step of staining cells in a sample obtained from a hematological sample by treatment with a hemolytic agent, with a fluorescent dye which can make a difference in the fluorescence intensity at least among mature leukocytes, leukocytes with abnormal DNA amount and immature leukocytes;

(2) a step of introducing the sample containing the stained cells into a flow cytometer to measure scattered light and fluorescence of the respective cells;

(3) a step of classifying leukocytes and coincidence cells/platelet clumps utilizing a difference in the intensity of a scattered light peak and a difference in the scattered light width;

(4) a step of classifying and counting mature leukocytes, leukocytes with abnormal DNA amount and immature leukocytes, utilizing a difference in the scattered light intensity and a difference in the fluorescence intensity of leukocytes classified in the step (3).

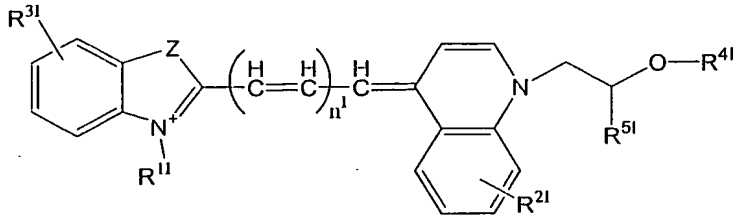
2. The method according to claim 1, which further comprises a step of calculating a ratio of mature leukocytes or immature leukocytes relative to leukocytes with abnormal DNA amount from a number of leukocytes with abnormal DNA amount and a number of mature leukocytes or immature leukocytes.

3. The method according to claim 1 or 2, which further comprises a step of calculating a ratio of immature leukocytes relative to mature leukocytes from a number of mature leukocytes and a number of immature leukocytes.

4. The method according to any one of claims 1 to 3, which comprises a step of further measuring a different kind of scattered light in the step (2), and classifying mature leukocytes into at least three groups and counting the same using the difference in the scattered light intensity and the difference in the fluorescence intensity of mature leukocytes obtained in the step (4).

5. The method according to any one of claims 1 to 4, which comprises a step of further measuring a different kind of scattered light in the step (2), and classifying immature leukocytes into at lease two groups and counting the same using the difference in the scattered light intensity and the difference in the fluorescence intensity of immature leukocytes obtained in the step (4).

6. The method according to any one of claims 1 to 5, wherein the fluorescent dye is selected from the group consisting of a compound represented by the formula (I):



(I)

(wherein R^{1I} is a hydrogen atom or a lower alkyl group; R^{2I} and R^{3I} each is a hydrogen atom, a lower alkyl group or a lower alkoxy group; R^{4I} is a hydrogen atom, an acyl group or a lower alkyl group; R^{5I} is a hydrogen atom or a lower alkyl group which may be substituted; Z is sulfur atom, oxygen atom, or carbon atom which is substituted by a lower alkyl group; n^I is 1 or 2; and X^{I-} is an anion), ethidium bromide, propidium iodide, ethidium-acridine heterodimer, ethidium azide, ethidium homodimer-1, ethidium homodimer-2, ethidium monoazide, TOTO-1, TO-PRO-1, TOTO-3, and TO-PRO-3.

7. The method according to any one of claims 1 to 6, wherein the hemolytic agent comprises the following components:

(1) a polyoxyethylene nonionic surfactant;

(2) a solubilizing agent to give damage to cell membrane of blood corpuscles and reduce their size;

(3) an amino acid; and

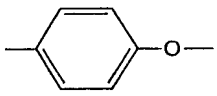
(4) a buffer by which pH and osmotic pressure of the liquid are adjusted to 5.0-9.0 and 150-600 mOsm/kg, respectively.

8. The method according to any one of claims 1 to 7, wherein the polyoxyethylene nonionic surfactant comprises a compound represented

by the following formula (II):

$$R^{1II}\text{-}R^{2II}\text{-}(CH_2CH_2O)n_{II}\text{-}H \qquad (II)$$

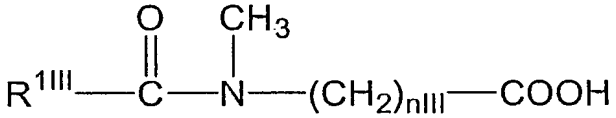
(wherein R^{1II} represents a $C_{9\text{-}25}$ alkyl, alkenyl or alkynyl group; R^{2II} represents -O-,



or -COO-; and n_{II} is 10-40).

9. The method according to any one of claims 1 to 8, wherein the solubilizing agent is a compound selected from the group consisting of

a sarcosine derivative of the formula (III):

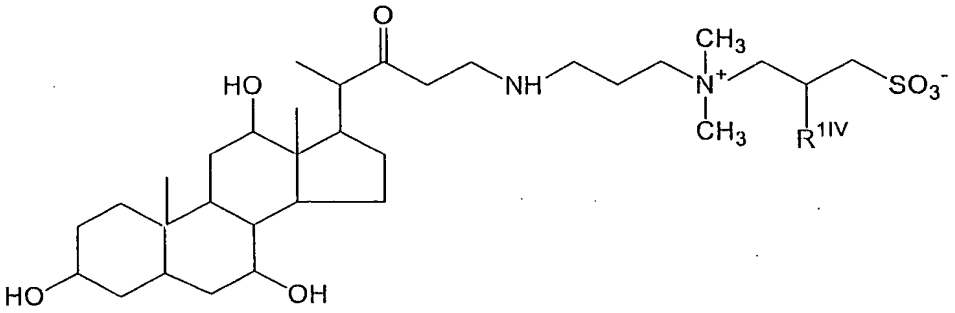


(III)

(wherein R^{1III} is a $C_{10\text{-}22}$ alkyl group; and n^{III} is 1-5)

or salts thereof;

a cholic acid derivative of the formula (IV):

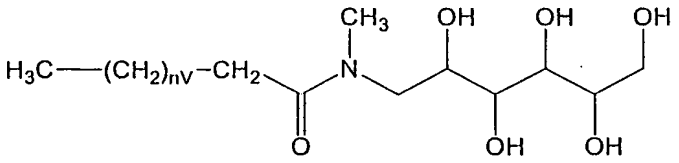


(IV)

(wherein R^{1IV} is a hydrogen atom or a hydroxy group);

and

a methylglucanamide of the formula (V):



(V)

(wherein n^V is 5-7).

10. The method according to any one of claims 1 to 9, wherein scattered light to be measured is selected from forward low angle scattered light, forward high angle scattered light and side scattered light.

11. A system for classifying and counting leukocytes which comprises:

a flow cytometer comprising an orifice portion in which a sample for measurement passes through, a light source which irradiates light to the orifice portion, a first light receiving portion which receives scattered light emitted from the orifice portion and a second light receiving portion which receives fluorescence emitted from the orifice portion, said sample for measurement being prepared by a step of mixing a hematological sample with a hemolytic agent and a step of staining cells in the resulting mixture with a fluorescent dye which can make a difference in the fluorescence intensity at least among mature leukocytes, leukocytes with abnormal DNA amount and immature leukocytes; and

an analyzing part in which the sample for measurement is analyzed by a step of classifying leukocytes and coincidence

cells/platelet clumps utilizing a difference in the intensity of a scattered light peak and a difference in the scattered light width and a step of classifying and counting mature leukocytes, leukocytes with abnormal DNA amount and immature leukocytes, utilizing a difference in the intensity and a difference in the fluorescence intensity of leukocytes classified.